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SEEDBORNE FUNGI AND VIRUSES IN BEAN CROPS (*Phaseolus vulgaris* L.) IN NICARAGUA AND TANZANIA

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ACADEMIC DISSERTATION

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ABBREVIATIONS

ANOVA	Analysis of Variance
BCMV	*Bean common mosaic virus*
BCMNV	*Bean common mosaic necrosis virus*
BLAST	Basic Local Alignment Search Tool
bp	Base pair
Cdna	Complementary DNA
CNIA	National Center of Agricultural Research
CMV	*Cucumber mosaic virus*
CB	Common bean
CPS	Capsular polysaccharide synthase
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide
dsRNA	Double-stranded RNA
DTT	Dithiothreitol
INTA	Nicaraguan Institute of Agricultural Technology
ITS	Internal transcribed spacer
M-MLV RT	Moloney Murine Leukemia Virus Reverse Transcriptase
MTR	Methyltransferase
NCBI	The National Center of Biotechnology
NIFAPRO	Nicaragua-Finland Agrobiotechnology Program
nt	Nucleotide
ORF	Open reading frame
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PvEV-1	*Phaseolus vulgaris* endornavirus 1
PvEV-2	*Phaseolus vulgaris* endornavirus 2
rDNA	Ribosomal DNA
Rrna	Ribosomal RNA
RdRp	RNA-dependent RNA polymerase
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SBMV	*Southern bean mosaic virus*
siRNA	Small interfering RNA
UGT	UDP-glycosyltransferase

CONTENTS

ABSTRACT

The common bean (*Phaseolus vulgaris* L.) is an important legume crop grown widely around the world due to its high nutritional values. In developing countries of Africa and Latin America (e.g. Tanzania and Nicaragua) bean crop is linked to food security and income generation especially in poorest groups made up of small farmers. Disease problems, pests, unimproved seeds, inappropriate agricultural management and environmental conditions are often the main constraints in bean crop production. In Nicaragua and Tanzania, the unavailability of certified seed of local bean varieties causes significant losses in yield and quality. Other limitation present in Nicaragua and Tanzania concerning bean diseases is that seed-borne fungi and viruses has gained little attention. Thus, for better understanding in seed-borne fungi and viruses associated with beans we investigated seed-borne fungi in an important new local bean variety. Sampling from four seed storehouses and six seed lots of cv. INTA Rojo was done in the main bean production areas in Nicaragua. In addition, to detect viruses infecting bean plants, we surveyed seedborne viruses in landraces and new common bean varieties introduced to Nicaragua (Central America) as well as improved varieties grown in Tanzania (eastern Africa).

The main results for seedborne fungi included 87 pathogenic isolates from eight genera: *Fusarium* spp. (*F. chlamydosporum*, *F. equiseti*, *F. incarnatum*), *Lasiodiplodia theobromae*, *Macrophomina phaseolina*, *Corynespora cassiicola*, *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, *Diaporthe* sp. (*Phomopsis*), *Aspergillus flavus*, and *Penicillium citrinum*. Subsequently, results based on germination in seed lots of common bean ('INTA Rojo') from four bean production areas in Nicaragua showed that germination was constantly less than 40% and could be as low as 16%, indicating disastrous yield losses for producers.

Four different species-specific primer pairs to detect *F. equiseti*, *F. chlamydosporum*, *F. incarnatum*, *C. capsici*, *C. gloesporiodes* and *C. cassiicola* were developed in this study based on sequence alignment of the internal transcribed spacer sequences (ITS1 and ITS2) from different fungi. They were tested in pure cultures of fungi and used successfully in detection of fungal pathogens from infected plants. These specific primers are able to give the basis to be used in seed health inspection (seeds and plants) for further research of the epidemiology, ecology, and control of the pathogenic fungi of common beans in the field.

The presence of *Phaseolus vulgaris* endornavirus 1 (PvEV-1) and PvEV-2 was detected in Nicaraguan and Tanzanian bean varieties. Likewise, *Cowpea mild mosaic virus* was detected in one region of Tanzania. There is

apparently indication that the new Nicaragua bean varieties are carrying virus resistance genes because no seedborne viruses were found in them.
In Tanzania improved protection against pathogenic seed-borne viruses is developed by resistance breeding.
These findings are the first report showing that several pathogenic seed-borne fungi occur in Nicaraguan beans. Previously, little information has been available on pathogenic fungi such as *F. equiseti*, *F. incarnatum*, *L. theobromae*, *C. cassiicola*, and *Diaporthe* spp in Nicaraguan common beans. This study has contributed in taking first steps to improve the pathological and genetic components in the national seed production system in Nicaragua and Tanzania by providing new knowledge concerning seed-borne pathogens associated with common bean.

RESUMEN

El frijol (*Phaseolus vulgaris* L.) es un cultivo leguminoso importante que crece extensivamente alrededor del mundo, debido a sus altos valores nutricionales. En países en desarrollo como África y América Latina (por ejemplo, Tanzania y Nicaragua), el cultivo del frijol está vinculado a la seguridad alimentaria y generación de ingresos especialmente en los grupos más pobres formados por pequeños productores. Problemas de enfermedades, plagas, semillas no mejoradas, inapropiado manejo agrícola y condiciones ambientales son a menudo las principales limitaciones en la producción del cultivo del frijol. En Nicaragua y Tanzania, la falta de uso de semilla libre de patógenos y la disponibilidad limitada de semillas certificadas de variedades locales de frijol requeridas para cubrir la demanda nacional para la producción del frijol, causan pérdidas significativas en el rendimiento y calidad. Otra limitación presente en Nicaragua y Tanzania con respecto a las enfermedades en el frijol es la poca atención que ha habido en la trasmisión de hongos y virus por semillas. Como resultado, hay muy pocos estudios que provean la seriedad de estos patógenos. Por lo tanto, para entender mejor la transmisión de hongos y virus por semillas en el frijol y obtener información, hemos investigado la transmisión de hongos por semilla en una nueva e importante variedad local INTA Rojo. Muestreo de cuatro almacenes y seis lotes de semillas de la variedad INTA Rojo fue realizado en las principales áreas de producción de semilla en Nicaragua. Además, se estudiaron virus trasmitidos por semillas en nuevas variedades mejoradas de frijol común introducidas en Nicaragua (América Central), variedades locales y variedades mejoradas cultivadas en Tanzania (África oriental) para conocer la incidencia y presencia de virus infectando el cultivo del frijol. Un set de cebadores específico de especie fue desarrollado basado en el alineamiento de secuencias de espacio transcritos internamente (ITS1 e ITS2) de diferente grupo de hongos. Estos cebadores moleculares fueron diseñados para ser usados en detectar hongos que infectan las plantas y para ser probados en cultivos puros de hongos. Además, estos cebadores serán utilizados en la inspección de semillas para controlar hongos patogénicos del frijol común en el campo.

En base a la morfología y el análisis molecular de las regiones ITS (ITS1 e ITS2) del gen ácido ribonucleico (ARN) ribosómico se identificaron diversos grupos de hongos. Los resultados principales para los hongos transmitidos por semilla incluyen 87 aislados patogénicos pertenecientes a ocho géneros: *Fusarium* spp. *(F. chlamydosporum*, *F. equiseti, F. incarnatum*), *Lasiodiplodia theobromae, Macrophomina phaseolina*, *Corynespora cassiicola*, *Colletotrichum capsisi*, *Colletotrichum gloeosporioides*, y *Diaporthe* sp. (Phomopsis), *Aspergillus flavus* y *Penicillium citrinum*.

Además, el estudio de investigación basado en los resultados de virus transmitidos por semillas indica la presencia del endornavirus 1 de *Phaseolus vulgaris* (PvEV-1) y el PvEV-2 en variedades de frijol. También se detectó el virus del mosaico leve del caupí en una región de Tanzania.

Estos resultados muestran que muchos hongos patogénicos ocurren en el frijol nicaragüense y que fueron reportados por primera vez en este estudio. Previamente, se disponía de poca información sobre hongos patogénicos tales como *F. equiseti*, *F. incarnatum*, *L. theobromae*, *C. cassiicola* y *Diaporthe* spp en el frijol en Nicaragua. Fue demostrado que *F. equiseti*, *F. chlamydosporum*, *F. incarnatum*, *C. capsici*, *C. gloesporiodes* y *C. cassiicola* pueden ser detectados usando un conjunto de cebadores específico desarrollados en este estudio en tejido del frijol. Además, aparentemente hay indicios de que las nuevas variedades de frijol de Nicaragua portan genes de resistencia a virus porque no se encontró ningún virus transmitido por semilla. En Tanzania, la mejora de la protección contra los virus transmitidos por semillas se desarrolla mediante la mejora de la resistencia. Este estudio proporciona nueva información y conocimiento sobre los patógenos transmitidos por semillas, especialmente los hongos patogénicos asociados al frijol común, y da los primeros pasos para cubrir los componentes patológicos y genéticos para mejorar el sistema nacional de producción de semillas en Nicaragua y Tanzania.

1. INTRODUCTION

1.1. General aspects of common bean

Common bean (*Phaseoulus vulgaris* L.) represents one of the most cultivated and edible legume in the world (Pachico 1989; Graham & Ranalli 1997; Broughton et al. 2003; Boye et al. 2010). In Nicaragua and Tanzania common bean (CB) for instance, represents the second most important crop after maize (Santalla et al. 1999; FAOSTAT 2014). Common bean, due to its nutritional properties (protein content, vitamins, zinc, iron and fiber), food security and income generation is considered as a crop with high economic importance in developing countries (Santala et al. 1999; Wortmann et al. 2004; Mora-Avilés et al. 2007; FAOSTAT 2014). Beans are herbaceous annuals plant that grown in tropical, subtropical and temperate zones with self-pollination while being alos able to out-cross (Free 1993; Ferreira et al. 2000; Gepts, 2001). The genetic diversity of bean is wide and very important. More than 90 % of the accessions found in the germplasm banks belong to the genus P. vulgaris (CIAT 2001). Common bean is originated from two centers of origins; the Andean area is characterized by large-seeded whereas Mesoamerican is characterized by small-seeded gene pools (Koenig & Gepts 1989; Graham & Ranalli 1997). Both morphological and molecular findings support these two centers of origin (Gepts et a.1986; Singh Nodari & Gepts1991; Singh et al. 1991; Becerra Velasquez & Gepts 1994; Blair et al. 2006).

The yield of common bean production in Latin America was estimated to be 11, 293,922 tons and Africa about 4,740,016 tons (FAO 2013; Jimenez 2014). Typically, common bean is grown by small-scale farmers. The yield is mainly consumed locally in diverse forms of traditional dishes (INTA 2002; INTA 2008). Common bean is cultivated under different cropping systems that includes mechanized, irrigated and intensive production. It is regularly intercropped with maize and sorghum (Schoonhoven & Voysest, 1991; Eden 2002; INTA 2002; INTA 2008).

In regions of Africa common bean can be grown twice a year while in Latin America, including Nicaragua, it is grown two to three times in the year (Belay 1998; INTA 2002; Wortmann et al. 2004 INTA 2008). However, in developing countries in east of Africa and Nicaragua most farmers get their seed from informal seed system that includes grains/seeds from local market as well as their own saved seeds/food from previous harvests (Van Gastel et al. 2002).

Generally, small-scale farmers are not always mindful the importance of high quality seed for production which includes genetic quality, pathological quality, physical quality and physiological quality (Louwaars 2007). Therefore, it is demonstrable that planting low quality of seed puts a risk for poor field emergence and non-vigorous plants (Matthews et al. 2012).

The importance of common bean as major food crop has been evaluated in many studies concerning the negative effects of quality of seeds, pests, diseases, climate change and intercrop system (Martinez et al. 2012). Infection by seed-borne pathogens may lower seed germination and emergence, result in stunted growth as well as into introduction of new diseases (Maddox 1998). Most of the pathogens causing diseases in common bean are seed-transmitted or can survive for long period in the soil and plant residues (Schwartz et al. 2005; Silvestro et al. 2013; Navas 2015; Lisboa et al. 2016).

Thus, the use of resistant cultivar and healthy seeds could be solution in combination with proper cultural practices for good management of bean pests and diseases (Singh 2000). Therefore, more studies are needed to improve common bean seed production (pathological quality) and to identify new sources of inoculum by controlling the occurrence of new pests and diseases.

1.2. Common bean in Nicaragua and Tanzania

In Nicaragua, while agriculture is the main economic activity based on small to medium scale farmers, it is also the sector most exposed to natural disasters. It commonly suffers from lack of access to technology, poor infrastructure for bean storage and inadequate resources. In Nicaragua basic grains are beans, maize (Zea mays L.), rice (Oriza sativa L.) and sorghum (Sorghum bicolor L.) that represent the basic diet routine for people. However, maize and bean are most important crops for food security and income generation (INTA 2002); FAOSTAT 2014).

Due to importance of beans, breeding programs are carried out to improve the cultivars and national seed production system (Jimenez 2014). Selected cultivars such as `INTA Rojo' and `INTA Cardenas' have been listed by the Nicaraguan government for extensive production in the cropping systems used by small-scale farmers. ‘INTA Rojo’ is highly relevant cultivar because of its characteristics which include high yield, good flavor, red skin color and drought tolerance (Jiménez et al. 2012; Jiménez & Korpelainen 2013).

Climate conditions promote the growth of beans throughout the year in primera (May-June), postrera (September-October) and apante (November-December) sowing seasons (INTA 2002; Gomez 2004; INTA 2009). There are four main production regions of common bean: I (Estelí), IV (Rivas, Granada and Carazo), VI (Matagalpa and Jinotega) and RAAS (Región Autónoma del Atlántico Sur, Nueva Guinea). In most of the regions of Nicaragua, common bean is grown in intercropping systems (e.g. with maize and sorghum) under conventional tillage (INTA 2002; INTA 2008).

The mean per capita consumption of bean in Nicaragua is 26.1 kg per year, but it will depend on yield, prices and exportations (IICA et al. 2009). Nevertheless, PESA/FAO & INTA/MAGFOR (2007) reported that the average of bean consumption per day was calculated to be 55.6 g, which represents 84.8% of the adequacy percentage of food sufficiency.

Despite efforts from farmers to produce beans there are several constraints that affect and limit bean production in Nicaragua. The main constraints include low-quality seed, non-certified seed, soil infertility due to nutritional deficiencies, deprived agronomic practices, water stress, lack of improved cultivars, pests and diseases. Infection with seed-borne pathogens can cause low germination and emergence rates, growth retardation, crop loss and as well as, introduction of new diseases (Maddox 1998; Solórzano & Malvick 2011). In response to these constraints, more research pointing to improve seed quality (seed health) to get more productivity and to develop diagnostic tools for seed health inspection and conduct studies concerning to the control of pathogenic fungi and virus of common beans in the field. These constraints in common bean production can force the governmental authorities to put more efforts in the phytosanitary regulation and to guarantee that only healthy seed can be marketed.

In Tanzania the common bean crop has been cultivated for home consumption, seed production in an artisanal way and well as for income security crop (Hillocks et al. 2006). Tanzania has been one of the major common bean producing country in East Africa. The importance of common bean in Tanzania is focusing on food/nutrition due to over 75% of rural households depend on beans for daily subsistence (Kalyebara and Buruchara 2008). In Tanzania, about 80% of rural community depend on agriculture for their livelihood (Nchimbi 1989). However, the main constraints in common bean are diseases, pests and abiotic factors. Major diseases (seed borne pathogens) are huge and the knowledge for viruses and fungi infection are low.

1.3. Major Bean diseases

Beans are vulnerable to several production constraints that include biotic and abiotic factors. Biotic constraints consisting of diseases caused by a wide range of pathogens (fungi, bacteria and viruses) and pests (nematodes, aphids, whiteflies and beetles) present a huge problem in bean cultivation. The most serious abiotic constraints are phosphorus and nitrogen deficiency, drought, poor soil fertility and low pH (Singh 1992; Agrios 1997; Opio et al. 2001; PROMESA 2002; Agrios 2005; Schwartz et al. 2005).

Among bacterial diseases the most common are bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) [(E. F. Smith) Dowson], bacterial brown spot (*Pseudomonas syringae* pv. *syringae*) and halo blight (*Pseudomonas syringae* pv. *phaseolicola*). These bacterial diseases affect different parts of common bean that include foliage (leave and stem), pods and seeds (Singh et al. 2010). Fungal diseases such as angular leaf spot (*Phaeoisariopsis griseola*), leaf rust (*Uromyces appendiculatus*) Pers) Unger, anthracnose (*Colletotrichum lindemuthianum*) Sacc & Magn), white mold caused by *Sclerotinia sclerotiorum* (Lib.), root rot (*Fusarium* spp and *Pythium* spp) are diseases with frequent

incidence and wide distribution in bean growing areas in America. (Schwartz et al. 2005; Hillocks et al. 2006; Singh 2010).

Other important diseases of common bean are those caused by viruses, *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) belonging from the genus *Potyvirus* and transmitted in bean seeds by aphids. Also, these viruses can be transmitted by pollen and mechanically.

Bean golden mosaic virus (BGMV) and *Bean golden yellow mosaic virus* (BGYMV) are diseases caused by begomoviruses which are transmitted by the whitefly *Bemisia tabaci* in a circulative persistent manner (Idris et al. 2001; Brown and Czosnek 2002). These diseases are considered among the most destructive plant viruses as they are responsible for severe losses in the tropical and subtropical regions of Central America, North America (United State and Mexico) and Caribbean (Agrios 1997; PROMESA 2002; Singh et al 2010).

Other bean disease present in Latin America is *Bean dwarf mosaic virus* transmitted in wild by *B. tabaci* in a persistent manner as well as by mechanical transmission.

Most of the major diseases affecting the commonly grown bean cultivars are seed transmitted. In view of the large number of potential bean diseases and their wide distribution around the world, it is evident that bean yield losses should be much higher in the absence of properly agricultural management practices. Some bacteria, viruses and fungi are transmitted by seed as a contaminant adhered to seed coat, or internally, which it is considered as the main mechanism of seed transmission (Waller et al. 2002).

Hence, the importance of good and an accurate diagnosis of plant bean diseases and their control by incorporating molecular tools and properly agricultural management to assure the food security, high production, pathogen-freed seed and good quality (Schwart et al. 2005).

1.4. Pathogenic fungi

Fungi are defined as “non-photosynthetic hyphal eukaryotes and related forms” (Carlile & Watkinson 1994). Most fungi are considered filamentous and reproduce by spores and/or have chitin and glucan containing cell walls. However, one large group of fungi, Deuteromycetes, do not form spores and these fungi are multiplied by growth of hyphal fragments. Fungi can be classified as obligate parasites (biotrophs), non-obligate parasites (necrotrophs and hemibiotrophs), facultative saprophytes or facultative parasites. There are more than 100 000 fungal species that are saprophytes but only 10 000 of them are known to cause diseases on plants (Agrios 1997).

Most of the airborne pathogens are biotrophic and produce wind-carried spores that infect aerial parts of the plant (Agrios 2005).

Plant infection by fungi is carried out by different steps: attachment (activity phase), germination (requires energy), appresorium formation (cell cycle progression, differentiated infection structures are used for penetration), penetration (start of the mechanical infection process and to facilitate enzymatic activity and osmotic pressure), invasive growth (high hyphae production) and finally sporulation (the cycle ends).Fungi use proteins in the infection process when they enter the cell (e.g. enzymes to breakdown cell wall). Hence, secretion of fungi play an important role in the pathogenesis because it may affect plant during the phase growth and development (Agrios 1997, 2005; Aro et al. 2005).

Most plant diseases are caused by fungi which are microscopic organisms producing hyphae that in masse form a mycelium. Mostly, these fungi are reproduced by spores. Spores are dispersal medium and regularly moved to the bean plant by soil, wind, water or other agents. In order to cause infection in the plant fungi need favorable conditions to survive. For instance, spore germination and the conduct of a pathogenic fungus in the nature will depend on the environmental factors. Under good environmental conditions, fungi can germinate to produce germ tubes and new hyphae can penetrate the plant throughout natural opening, wounds or surface of the host. In many plant diseases, fungal pathogens can show their signs of infection by producing structures on the surface of the host. For instance, these structures include spores, mycelia and sclerotia.

There is a large list of fungal symptoms that include cankers, leaf spot, necrosis, rots, anthracnose, and decline, death of the plant and visible structure of sclerotia, mycelium and spore on the plant (Agrios 1997; Kronstad 2000; Agrios 2005; Porch et al. 2014). Necrotic symptoms consist of leaf spots, blight, root rot, canker, scab, damping-off, soft stem rot and anthracnose. Other symptoms caused by fungi are wilting, rust, smut and mildew (Agrios 1997, 2005).

1.4.1. Seedborne pathogenic fungi associated with common bean in Nicaragua

Pathogenic fungi have a great ability to infect seeds internally and externally. When the focus of infection is in the inner seed, the damage is more serious, as it may destroy the embryo. Furthermore, seed-borne fungi can contaminate the seeds and cause in bean cultivars yield loss, affect seed quality, seedling germination and growth. Seed-borne pathogenic fungi also can cause diseases on roots, in aerial parts of bean and in other underground body. These fungi can also produce toxins making the beans inappropriate for consumption (Waller et al 2002; Makun et al. 2009; Makun et al. 2010; Narayan et al. 2013; Amodu and Aku 2015; Z.I. El-Gali 2015).

Thus, seed-borne fungi are mainly the causal agents of root diseases in beans and represent the main biotic component of yield decline in an intensively cultivated area. Seed-borne fungi can survive in soils for long period and cause root rot with influence ranging in severity from declined growth rates to the death of the plant, depending on the host susceptibility and environmental conditions. Fungal pathogens causing root rot can survive in soil and colonize roots of host crops in the mycelium state. In fact, temperature of soil is the main driving force of their development and the physiochemical factor keys of their ecology (Hall 1995).

Some examples of seedborne fungi are *Pythium* spp., *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani,* Kühn., *Fusarium oxysporum* (Schltdl.) Fr., *F.* solani (Mart.) Sacc., and *Colletotrichum lindemuthianum* (Sacc. & Magnus) which are potential pathogens causing serious economic damages in bean to limit production to a large extent and in a catastrophic manner for farmers.

Recently, seed-borne pathogens of common bean in Nicaragua were detected and reported. The method used for fungi detection was incubation tests (MAGFOR-OIRSA 2004). The fungi detected are listed in Table 1.

Table 1. Pathogens identified in bean seeds in Nicaragua (MAGFOR, 2004)

Fungi transmitted on seed coat	**Fungi transmitted internally in embryo**	**Bacteria**	**Viruses**
Alternaria alternata	*Fusarium oxysporum.*	*Burkholderia phaseoli*	*Bean common mosaic virus*
A. tenuis	*F. parasiticum*	*Burkholderia syringae pv. oli*	
Botryodiplodia sp.	*Fusarium f. sp. phaseoli*		
Colletotrichum lindemuthianum	*F. ramularia*		
Curvalaria sp.	*F. phaseolina*		
Diplodia sp.	*F. vasinfectum*		
Entyloma petunia	*Pythium aphanidermatium*		
Erysiphe poligoni			
Macrophomina phaseolina			
Phyllosticta phaseolina			

The main recently seed-borne fungi known to infect common bean in Nicaragua is reported in this study.

1.5. Plant Viruses

Plant virus diseases can cause extremely serious damages leading to high yield losses in many crops grown worldwide. However, yield loss by viruses depend on several factors: transmission system (seed, pollen, fungi, vector or mechanically), hosts (for instance, crop variety), crop system and virulence evolution (Agrios 1997). Agrios (1997) defined a virus “as nucleoprotein” with the competence to cause disease on the host. Viruses as plant pathogens are considered as intracellular parasites with a small genome (RNA or DNA) that contain only few genes. Mostly, the spreading of plant viruses from plant to plant is by arthropod vectors and the potential to cause damages depends on the initial number of infected plants (Maule & Wang 1996).

Some viruses are transmitted by seeds. Seed-borne virus infections can be catastrophic as the plant is affected from the beginning of growth phase. Viruses cannot be controlled with any chemical treatment in the field, and as a result, its control must be based on measures to prevent infection. On the contrary, if the infection occurs later, for instance in the end of the growing season yield losses are often smaller. Thus, yield losses might be reduced using appropriately agricultural practices that delay virus spread. Nevertheless, the most important principle for controlling viruses is to use constantly virus-free planting material. In the field, viruses can spread from plant to plant only by help of a vector or through contact between plants. Some species such as insects, nematodes and soil fungi transmit plant viruses. Acquisition and transmission of viruses can occur very quickly requiring only seconds, minutes and hours to cause virus infection. Single virus-carrying insect, nematode or fungal zoospore is enough for initiating virus infection. Therefore, control of vectors using pesticides provides only limited or no success in virus control and in addition can provoke damages in the environment. If virus infects the first cell, it can multiply and moves to neighboring cells and other parts of the plant, including roots, leaves, fruit and occasionally seeds. Virus infection in plants, including common bean, may cause growth reduction, stunting, mosaic, yellowing malformation and ring spots (Figure 2). After a week or two from infection, infected plant itself will be a virus source, from which vectors can transmit the virus to the neighboring plants. Some viruses remain infectious in their vector through the vector’s lifetime and which can be transmitted over thousands of kilometers if wind-borne migration of the vector occurs. Virus-carrying insects and fungal resting spores can be dispersed over long distances by storms (Agrios 1997; Agrios 2005).

1.5.1. Virus diseases associated with common bean in Nicaragua and Tanzania

Bean diseases caused by viruses are considered one the major diseases affecting bean crops worldwide. In Nicaragua, very little information is known on the occurrence and epidemiology of bean viruses, especially those concerning seed transmission. However, in Central America it has been reported and identified seven viruses that infect beans: *Bean common mosaic virus* (BCMV); *Bean golden mosaic virus* (BGMV); *Bean mild mosaic virus* (BMMV); *Bean rugose mosaic virus* (BRMV); *Bean chlorotic mottle virus* (BClMV*); Quail pea mosaic virus* (QPMV) *and Sourthen bean mosaic virus* (SBMV). However, only *BCMV* and *SBMV* were reported in Nicaragua by Gamez (1973) and Fuentes & Anderson (1990). Rojas (1998), detected for the first-time occurrence of *Bean yellow mosaic virus* (BYMV) and *BCMV* in landraces and improved varieties of common bean in Nicaragua. Also, diseases caused by whitefly (*Bemisia tabaci*) transmitted begomoviruses (family Geminiviridae; genus Begomovirus) are considered important pathogens in many hosts that include dicotyledonous bean plants and vegetables (Morales, 2001; Singh & Schwartz, 2010). Begomoviruses are one of the main constrains in Nicaraguan bean production. Early infection by begomoviruses can cause a total crop loss (Rojas 2004). The often genome contains single stranded-circular DNA but also many begomoviruses have a bipartite genome. The bipartite genome consists of DNA-A and DNA-B component where the size of each one is calculated about 2.6-2.7 kb (Ala-Poikela et al. 2005). In an extensive study concerning begomoviruses, Karkashian et al (2011) detected *Bean golden yellow mosaic virus* (BGYMV) in Nicaragua. Typical symptoms of begomovirus infection in common bean (Figure 2) include stunting, curling leaves, mosaic, yellowing of leaves, reduced growth and malformation-distortion of pods (Lapidot & Friedmann, 2000; Bracero & Rivera, 2003).

Viruses have been reported to cause infection in common bean crops. Among these viruses are *Bean Common Mosaic Virus* (BCMV) and *Bean common mosaic necrosis virus (*BCMNV) from genus Potyvirus. *Cowpea mild mottle virus* (CPMMV) from genus *Carlavirus, Southern bean mosaic virus* (SBMV), *Cucumber mosaic virus* (CMV) from genus *Cucumovirus*; family Bromoviridae., *Phaseolus vulgaris* endornavirus 1 (PvEV-1) and *Phaseolus vulgaris* endornavirus 2 (PvEV-2)., *Bean golden yellow mosaic virus* (BGYMV), begomovirus. Some of these viruses have been detected in Nicaragua as mentioned before (Schwartz et al. 2005; Mwaipopo et al.2017).

The major virus diseases on common bean are those transmitted via seeds. Transmission of BCMV and BCMNV is considered as via seeds. The transmission of these viruses may also occur by aphids during growing season (Morales & Castaño 1987; Schwartz et al. 2005; Galvez 1980). BCMV and BCMNV cause yield losses in many important areas of bean production. Bean plants affected by BCMV and BCMNV show symptoms such as leaf distortion,

necrotic local lesion, necrotic root, mild, light or fark green mosaic (Morales & Bos 1988; Schwartz et al. 2005; Mwaipopo et al.2017).

Southern bean mosaic virus (SBMV) was reported in Nicaragua by Gamez (1973) and Fuentes & Anderson (1990) causing mild, mosaic and mottle symptoms on common bean (Schwartz et al. 2005; Mwaipopo et al.2017). Yield losses can reach up to 50 %. SBMV is transmitted by beetles (*Chrysomelidae*) and via seeds with a range of 1 to 5 % (Hull 2004; Schwartz et al. 2005).

Cucumber mosaic virus (CMV) is a seedborne virus infecting more than 1200 host species in over 100 families of monocotyledonous and dicotyledonous (Palukaitis & García-Arenal 2003; Ziter & Murphy 2009). This virus can be transmitted by more than 80 aphid species in non-persistent manner. CMV transmission by aphid is depending on virus strain, species of aphid and the age or growth stage of the host plant. Not all strains of CMV can be transmitted by aphids (Gallitelli 2000). CMV on common bean crops cause some symptoms that comprise common mosaic, chlorosis and dark green vein banding, flower abortion and abnormal development (Bos & Maat 1974; David & Hampton 1986; Schwartz et al. 2005; Zitter & Murphy 2009).

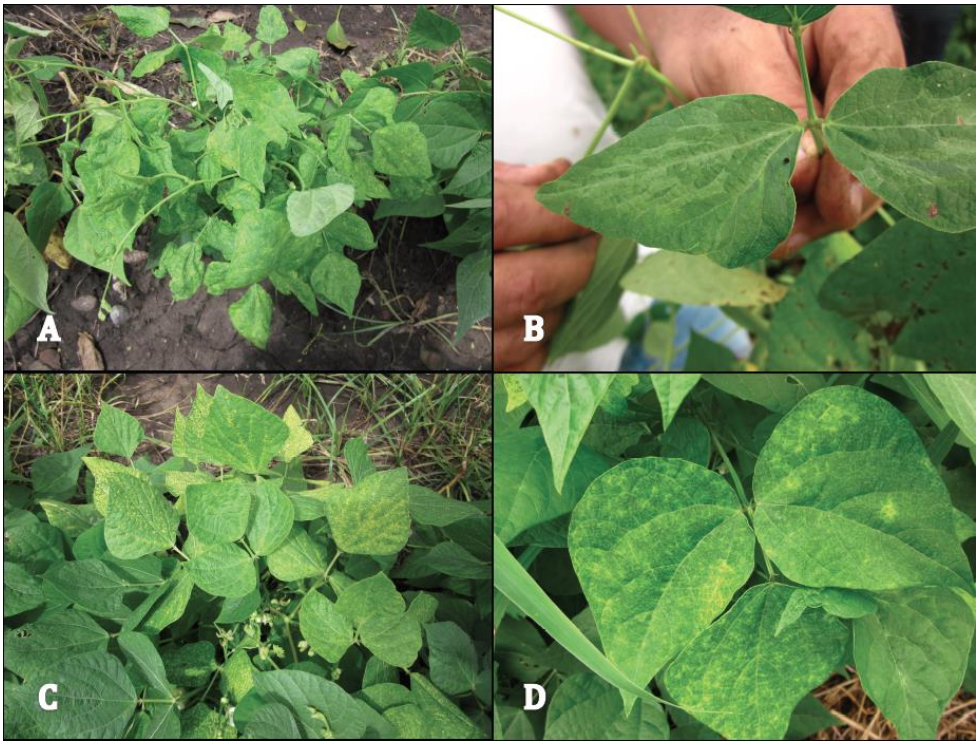

Figure 1. Virus symptoms observed in common bean plants in La Compañia, Nicaragua. (a), Stunting of the plant, malformation and blistering of leaves. (b), Mild epinasty and vein reversion. (c), Green-yellow chlorosis. (d), Green-yellow mosaic.

Figure 2. Bean plant (cv. INTA Rojo) from Boaco with mosaic, yellowing and curling of leaves.

In Tanzania common bean is infected by huge range of viruses that include single- and double-stranded DNA and RNA plant viruses. Among these viral diseases are BCMNV and BCMV which are viruses transmitted by seeds. Other viruses infecting and reported in common bean are CPMMV, CMV, SBMV, BGYMV, SYMMoV Calopogonium golden mosaic virus (CalGMV) and endornaviruses PvEV-1 and PvEV-2 (Mwaipopo et al. 2017; Karkashian et al. 2011).

1.6. Management strategies for bean diseases

The harmful seed-borne pathogens identified in common bean in Nicaragua indicate that seed health is a concern for governmental authorities especially to the regulating entity of seed production system. In spite of the economic importance of common bean diseases, effective methods to control seed-borne pathogens are lacking. There are different approaches that may be used to avoid, mitigate or control bean diseases. Within the approaches, the good agronomic management of the crop is very important because it includes cultural practices that control the spread of bean diseases and improve yield. Among these agricultural practices include resistant varieties, crop rotation, biological control, the use of modified substrate with compost, chemical control, biological and chemical fertilization.

These management strategies for seed and soil-borne pathogens are shown to suppress bean diseases (INTA 2002; INTA 2009; Hall 1996).

2. AIMS OF THIS STUDY

The overall aim of this study was to identify seedborne pathogens associated with common bean (*Phaseolus vulgaris* L) in Nicaragua and Tanzania. The specific objectives of the present study were as follow:

1. To identify fungi transmitted in the bean seeds ('INTA Rojo') and to test their pathogenicity on seedlings.

2. To develop species-specific primers for detection of seedborne pathogenic fungi that infect common bean in Nicaragua.

3. To advance knowledge on the occurrence of seedborne viruses in common bean varieties currently grown in Nicaragua and Tanzania.

3. MATERIALS AND METHODS

3.1. Survey and sampling of plant and seed material

For the seed-borne fungi detection study (sub-study I), seeds of common bean were sampled and chosen from four seed storehouses and six seed lots of cv. INTA Rojo harvested from Boaco, Carazo, Estelí, and Matagalpa, representing the main bean growing areas in Nicaragua (Figure 3).

In the sub-study I, lima bean (*P. lunatus* L.) obtained from the former MTT Agrifood Research Finland currently Natural Resources Institute) was tested for pathogenicity tests. More details about pathogenicity, surveys and sampling of bean seeds can be found in the sub-study I.

Seeds of three different common bean varieties (INTA Rojo, SEN 46, and INTA Cárdenas) from Carazo and a variety of faba bean (*Vicia faba* L. Threefold White) in addition with seeds of tomato (Tomat Dansk Export II), pepper (Redskin) and squash (Black Forest F1) obtained from Finland were tested for *F. oxysporum* pathogenicity test (sub-study II).

Different common bean varieties were surveyed and sampled in the field of La Compañia located in Carazo during the first cropping season of the year called primera (May-August). Leaves and seeds from bean plants were collected for small interfering RNA (siRNA)-based virus detection (sub-study III). These varieties include SEN46 (released as INTA Caribe), SEN52 (released as INTA Negro Precoz), CENTA Pipil and MIB396 (released as INTA Nutritivo).

For detection of *Bean golden mosaic virus* (genus Begomovirus; Geminiviridae) in the sub-study III, leaves of common bean were collected from recently released common bean varieties grown in La Compañia, including ‘INTA Cárdenas’, ‘INTA Rojo’, ‘INTA Seda 2’ (breeding line with code NIC-704), ‘INTA Fuerte Sequia’ (RS-811-22), ‘INTA Frijol Norte' (628-SM-22-2), and the breeding line XRAV-404.

In Tanzania, collection of samples was focused on 38 common bean landraces grown in three different areas (Southern highland, Eastern and Northern zones). The collected plants were grown in pots in greenhouses either at Sokoine University of Agriculture or at Mikocheni Agricultural Research Institute. The plant material was taken from 30 common bean plants per area and directly subjected to RNA isolation (sub-study III).

Figure. 3. Common bean sampling areas in Nicaragua 2008. I (Estelí), IV (Carazo); VI (Matagalpa) and V (Boaco). Nicaragua Map downloaded and adapted from Google map (2017).

3.2. Methods

3.2.1. Experiments in the laboratory

For seed-borne fungi detection, total DNA from pure fungal cultural growing on potato dextrose agar (PDA) was isolated as described in the sub-study I and II. Identification of fungi was based on the sequences of the polymerase chain reaction (PCR) products of fungal ITS regions amplified with molecular primers ITS1 and ITS4 (sub-study I, III). The amplified PCR product contains ITS1 and ITS2 regions neighboring sites of 18S and 28S rRNA (White at al. 1990).
Genomic DNA and RNA extractions method for virus detection is showed in a detail in the paper III. PCR reactions methods and primers used for fungi and virus detection are shown in the sub-studies (I, II, III).
For virus detection on bean plants, equal amounts of total RNA from diverse samples were pooled (30–36 samples per pool), and small RNAs were exposed to deep sequencing (Illumina). Assembly of the reads (21, 22 and 24 nt) to contiguous sequences and searches for homologous viral sequences in databases was carried out. The small-RNA reads from each pool of samples were mapped. More description is found in the sub-study III.

3.2.2. Analysis of emergence and symptoms of seedlings

For testing emergence and symptoms of bean seedlings, eight sub-samples (50 beans each) were taken from each of the six samples. Each subsample was planted in a separate tray filled with sterilized washed sand and peat. The trays were arranged according to a completely randomized design in a growth room (20–22 °C) in dim light (photoperiod 11 h). Emergence of seedlings was observed for 15 days to evaluate and determine symptoms of diseases in the stem base and roots. More details are described in the sub-study I.

3.2.3. Isolation of fungi and fungal material

In total, 133 fungal isolates were obtained from surface-sterilized beans of the six seed lots and tested for pathogenicity on lima beans. However, based on the pathogenicity tests results, only 87 of them are associated with symptoms of seed-borne fungi complex on bean plant (sub-study I).

In the laboratory, fungal material growing from the surface of bean seeds were taken and placed into Petri dishes containing fresh culture medium, potato dextrose agar (PDA) complemented with streptomycin (Sigma) at 50 mg/l. The Petri dishes were incubated at room temperature (25–30 °C) in the dark for 4–7 days. Fungal material was refreshing and keeping in Petri dishes in cold room (5 °C; sub-study I, II).

3.2.4. Pathogenicity tests

Pathogenicity of 113 fungal isolates on beans was measured twice in two independent experiments. There were four replicates and one non-inoculated control (healthy lima bean, *P. lunatus* L.). The pathogenicity was done following the protocol of the reference (Lehtonen et al. 2008). Pathogenicity of the fungal isolates was evaluated 20 days post-inoculation (dpi).

To fulfill Koch's postulates, pieces of symptomatic tissue were removed from the seedlings with a sterile scalpel, transferred to PDA, and fungal growth was monitored and identified by using the microscope (sub-studies I, III).

Besides, pathogenicity test for 11 *F. oxysporum* isolates on beans was assessed in three independent experiments using the method described by Lehtonen et al. 2008 (sub-study I). Additionally, pathogenicity of 11 *F. oxysporum* isolates was tested on three different vegetable cultivars from a horticultural shop (K-RAUTA, Helsinki, Finland). Seeds of tomato (Tomat Dansk Export II), pepper (Redskin) and squash (Black Forest F1) were surface sterilized and germinated on moist sterile filter paper in Petri dishes. We used four replicates and a non-inoculated control were used per each *F. oxysporum* isolate and crop species. After 14 days of germination and emergence, 150 seedlings were transplanted into a tray filled with sand (sub-study II).

3.2.5. Phylogenetic analysis of sequences

For phylogenetic analysis from fungal isolates (Boaco and Carazo), ITS1 and ITS2 sequences (~450 nt) from *Lasiodiplodia theobromae* were aligned using Clustalw (Thompson et al. 1994) and subjected to phylogenetic analyses. Neighbor-joining method was implemented using 1000 replicates and the Kimura two-parameter model as implemented in MEGA version 5.1 (Tamura et al. 2007).

For endornaviruses sequences, phylogenetic analysis was carried out taking sequences of the helicase 1–encoding region in PvEV-1 and PvEV-2 and aligned with the helicase protein sequences of some other endornaviruses retrieved from GenBank databases. The neighbor-joining method was used. Likewise, DNA sequences of begomoviruses were submitted to analysis with BLAST (ttp://blast.nbeani.nlm.nih.gov/Blast.cgi), aligned with similar sequences found in NCBI/GenBank and subjected to phylogenetic analysis. Neighbor-joining tree was calculated using MEGA 5.1. The description of phylogenetic analysis process is explained in detail in the sub-studies I and III.

3.2.6 Statiscal analyses

The data from the experiment testing the emergence and symptoms in seedlings (sub-study I), was carried out using One-way analysis of variance (ANOVA) and also using the comparison of means based on the Tukey test (α =0.05) to determine if the bean seed lots differed with respect to emergence and incidence of disease-like symptoms (sub-study I). The experiment was organized based on Completely Random Design (CRD) using the six seed lots and eight repetitions of each.

The materials and methods used in this study are summarized in Table2:

Table 2. Materials and methods used in this study

Materials and methods	*Publications*
Seed material	I, II, III
Sampling (Figure 3)	I, II, III
Isolation and identification of seed-borne of fungi	I, II
Fungal isolates	I, II
Helicase sequences of endornaviruses	I, II, III
Genomic DNA extraction	I, II, III
Amplification (PCR) and sequencing of the ITS rDNA	I, II
Pathogenicity test	I, II
Phylogenetic analysis	I, III
RNA isolation	III
RNA amplification	III
Virus detection by siRNA sequencing and data analysis	III
Virus detection by RT-PCR	III
Primer design	II
Greenhouse experiment	I, III
Growth chamber	II

4. RESULTS AND DISCUSSION

4.1. Identification of seedborne pathogenic fungi in common bean cv. INTA Rojo and pathogenicity of fungal isolates (sub-study I)

Our approach in this study was to identify seed-borne fungi and their pathogenicity in an important cultivar (INTA Rojo) in Nicaragua. To gain knowledge of potential harmful fungi infecting bean crop, bean seeds contamination and the pathogenicity of the isolates fungi from different regions were examined. The fungi are transmitted as contaminants adhered to seed coat, or internally which is considered as main mechanism of seed-mediated transmission. Seed-borne fungi is known to reduce germination, emergence, growth, and yield in bean seeds. However, when the infection is focusing on beans as grains used for food can reduce the nutritional value or in most cases can produce toxins making the beans not suitable for consumption (Narayan et al. 2013; Waller et al. 2002).

A total of 133 isolates of seed-borne fungi from surface-sterilized beans of six seed lots were obtained. Phenotypically similar fungal isolates were placed to the same group (designated as a 'phenogroup') based on the morphological and growth characteristics.

Our results from analysis of emergence and symptoms of seedlings (Table 1, sub-study I) demonstrated that germination in seed lots of common bean ('INTA Rojo') from four important bean production areas sampled in Nicaragua was continuously less than 40% and as low as 16%, what turns out to be catastrophic for the farmers. However, results from those seed lots that showed better emergence provided rise to a larger proportion of healthy seedlings, whereas poor emergence was associated with abnormal growth (poor growth), and disease-like symptoms caused by pathogenic fungi (Figure 2, sub-study I).

Afterwards, 133 these fungal isolates were tested for pathogenicity on lima beans in two independent experiments, and the results in both experiments were consistent in showing that 87 fungal isolates were pathogenic, i.e. caused symptoms on bean seedlings (Figures 3 and 4, sub-study I). Only pathogenic isolates were characterized on the basis molecular analysis and identified morphologically to the genus level. Eight distinguishable phenogroups were identified. Description and characteristic of these eight groups of fungi are presented in more detail in Table 2 sub-study I.

The morphological identification of seed-borne pathogenic fungi was reliable with analysis of ITS (TS1 and ITS2) sequences which make it a useful combination tool. Universal ITS primers (ITS1 and ITS4) are used to amplify a large range of fungi (White et al. 1990; Anderson & Parkin 2007), which allow to identify the fungal species based on inter- and intraspecific variations observed in the ITS regions between fungal species (Terashima et al. 2002).

Among seedborne pathogenic fungi, the genera identified were *Fusarium* spp (F. *chlamydosporum, F. equiseti* and *F. incarnatum*), *Macrophomina phaseolina*, *Lasiodiplodia theobromae, Corynespora. Cassiicola*, *Colletotrichum* spp (*C. capsici* and *C. gloeosporioides)*, *Penicillium citrinum*, *Aspergillus flavus* and *Diaporthe* sp (*Phomopsi*s). These pathogenic fungi found in our study have been recorded from seeds of legumes including bean and other crops as saprobes, facultative parasites, cosmopolitan endophytes or pathogens causing damages to seed and seedlings in different countries (Punithalingan 1980; Navas & Subero 1995; Rusuku et al.1997; Maia et al. 2004; Agrios 2005; Farr et al. 2006; Udayanga et al. 2011; Amaike and Keller 2011; Gupta et al. 2012; Lima et al. 2013; Silvestro et al 2013; Gomes et al. 2013; Machado et al. 2014; Kumar 2014; Henrique et al 2014; Dugan et al. 2014; Farr and Rosmman 2015; dos Santos et al. 2015; Thompson et al. 2015; Rosado et al. 2016; Lisboa et al. 2016).

The highest incidence and most common pathogenic fungi isolates were *Fusarium* spp *(Fusarium chamydosporum, F. equiseti and F. incarnatum)* phenogroup I*, L. theobromae, P. citrinum*, and *M. phaseolina*.

Seedborne fungi of common bean displayed variable effects on seedlings and based on the results of pathogenicity tests, pathogenic fungi were grouped into eight phenogroups: Phenogroup I, *Fusarium* spp (*F. chlamydosporum, F. equiseti* and *F. incarnatum*) caused visible damages in all the inoculated seedlings. *Fusarium* spp. caused root rot, lesions on the stem and poor growth of seedlings. This fungus has a wide host range and has been detected in many crops (Agrios 2005). However, certain species of *Fusarium* spp. have been used as biocontrol agents (Hall 1996). Fungi in the phenogroup II (*M. phaseolina*) induced typical characteristic symptoms of charcoal rot, dark lesions on stems, and root rot. Among isolates of phenogroup III (*Lasiodiplodia theobromae*) and IV (*Corynespora cassiicola*) induced severe symptoms including dieback, decay and cankers on stems and roots of the inoculated bean seedlings. The phenogroup V (*Colletotrichum*) induced in general characteristics symptoms of anthracnose. *C. capsici* include small dark spot on the stem, discoloration of roots and dark spots on the cotyledon whereas *C. gloesporiodes* caused small lesions dark brown to black, respectively. The isolates of the phenogroups VI and VII (*Penicillium citrinum* and *Aspergillus flavus*, respectively) caused simply mild symptoms such as discoloration on the stem of inoculated bean seedlings. Phenogroup VIII (*Diaporth*e sp), caused canker and decay symptoms. The findings from this study indicate the potential damage that can be incurred by seed-borne pathogenic fungi on Nicaraguan bean cultivars and food security.

Fusarium spp (**phenogroup I**). were detected in seed lots in all four regions surveyed in Nicaragua. *Fusarium* species are soil-borne fungi that can cause rot of the root, stem, and fruit or vascular wilt in a wide range of crop plants, and they survive as saprophytes (Silvestro et al. 2013). The taxonomic complexity of *Fusarium* spp. is due to the wide range of different races and makes it more

difficult to control (Henrique et al. 2014). *Fusarium* spp. is known to produce mycotoxin which is a concern to human and animal health in many field crops, including common beans (Agrios 2005; Tseng et al. 1995; Van Diepeningen & Sybren de Hoog 2016).

There is limited previous information about seed-borne infections of *F. incarnatum* in common beans or its pathogenicity on common bean seedlings. Nonetheless, *F. equiseti* is known to infect many forms of legumes crops including bush bean (*L. lunatus*), kidney bean and haricot bean *(L. vulgaris*) as well as faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.), lentil (*Lens culinaris* L.), cowpea (*Vigna unguiculata* L.), soybean (*Glycine max* L. (Merr) and mung bean (*Vigna radiata* (L.) R. Wilczek.] [Chaudhary & Kaur 2002; Rodrigues & Menezes 2005]. In cowpea crop, *F. equiseti* causes necrosis on the plant top and the disease progresses to the rest of the plant including stem, which can result in plant death (Rodrigues & Menezes 2005). *F. equiseti* occurs mainly in tropical and subtropical regions, but it has been found also in temperate areas in Europe and North America (Booth 1978, Bosch & Mirocha 1992). *F. equiseti* is a fungus an often found in soil and straw mulch in ginseng (*Pana ginseng* M.) fields in British Columbia causing symptoms of reddish brown discoloration on ginseng roots (Punja et al. 2007).

Ginseng plants affected by *F. equiseti* show seed decay and reddish brown to black lesions on hypocotyls and roots. *F. equiseti* shows high adaptability in many cropping systems (Goswami et al. 2008) and is capable of infecting seeds, roots, tubers and fruits. Besides, this fungus has been isolated and reported in cotton (*Gossypium hirsutum* L.) [Chimbekujwo 2000], sugar beet (*Beta vulgaris*) [Stojsin et al. 2000], potato (*Solanum tuberosum* L.; Theron & Holtz 1989) and pine (*Pinus* sp) (Ocamp & Juzwik 1995). The reports mentioned previously showed that several leguminous species are susceptible to infection by *F. equiseti.* However, report of *F. equiseti* infecting specifically common bean was not available yet. Our study seems to be one of the first in which *F. equiseti* is implicated as seedborne pathogenic fungus in common bean.

F. incarnatum is a fungus not reported commonly but for the first time it was found to infect *Capsicum annum* L. (Ramdial et al. 2016) and *Ziziphus jujube* Mill. (Guo et al. 2016).

F. chlamydosporum, has been reported to be isolated from soil, beans, and maize roots in Kenya (Okoth et al. 2010). Plant diseases caused by *Fusarium* spp. are one of the major and more catastrophic diseases in bean crops. *Fusarium* spp. has to be reported affecting common bean production in the Central American region. For instance, in Cuba, half of the seed lots surveyed for fungi were found to contain *Fusarium* spp. (Martínez et al 2014), including *F. solani* f. sp. *phaseoli* causing substantial yield losses in common bean crops in many regions of Mexico (Martínez-Garnica et al. 2014).

M. phaseolina was detected (**phenogroup II)** in beans in three seed lots in the surveyed regions. *M. phaseolina* is one of the most important pathogens of common bean plants and it's considered a polyphagous pathogen because it does show host specificity (Maia et al. 2004; Gupta et al. 2012). Our results (sub-study I) from phylogenetic analysis showed three clades. The first one contained five Nicaraguan fungal isolates belonged to *M. phaseolina* described from other isolates such as: DTMp3 (isolated from *Vigna radiata* L. in China); IFAPA-CH724 (isolated from *Fragaria x ananassa* in Spain); K4223 (isolated from *Pisum sativum* in South of Australia); MP76 (isolated from *Fraxinu*s sp in USA). The additional clades included fungi such as *Botrysphaeria mamame*, and *B. dothidea* (data not shown). These results are consistent with earlier reported variation in morphology and virulence among isolates of *M. phaseolina* in plants comprising common bean, soybean, and other crops (Dhingra et al. 1972; Echavez-Badel et al. 1991).

According to Su et al. (2000) the host specialization of *M. phaseolina* is apparent in corn but not in sorghum, cotton, or soybean.

The disease caused by *M. phaseolina* is known as ashy stem blight or charcoal rot. This fungus survives in the soil as microesclerotia and on infected plant debris. Infected seeds by *M. phaseolina* do not germinate or produce seedlings which died soon after emergence. Microesclerotia are black and serve as source of inoculum under unfavorable conditions that include low levels of soil nutrients, high temperatures, hot and dry weather. These environmental conditions promote infection and development of charcoal rot. High populations of *M. phaseolina* in the soil may be developed when the host is susceptible and cropped in consecutive years and the pathogen is redistributed by tillage practices. In good favorable environmental conditions *M. phaseolina* causes diseases such as damping-off, seedling blight, collar rot, stem rots, root rots and charcoal rots in a large range of an important edible plants, as mentioned above. In fact, based on previous study of

M. phaseolina in common bean demonstrated that salinity as stimuli to plant infection and increase severity of disease. In other words, *M. phaseolina* can be more severe disease if *P. vulgaris* is growing in saline soils (Pei You et al. 2011).

Molecular characterization of *M. phaseolina* using PCR-based genomic fingerprinting techniques, such as PCR-restriction fragment length polymorphism (RFLP) of the ITS region and random amplified polymorphic DNA (RAPD) have contributed to understanding of the population biology of this fungus isolated from soybean, cotton and sorghum (Almeida et al. 2003). Purkayastha et al. (2006) characterized different isolates of *M. phaseolina* collected from several host species growing in or near by cluster bean (*Cyamopsis tetragonoba*) fields using conventional and molecular techniques. They found that all the fungal isolates were pathogenic to cluster bean.

Symptoms appeared include premature yellowing of the top leaves, premature leaf drop, and discoloration of stem and roots from silver to light grey. Black sclerotia were visible as typical with charcoal rots.

Results of the pathogenicity tests carried out in this study were relatively similar those reported by Purkayastha et al. (2006) who showed that 59 isolates of *M. phaseolina* collected from different hosts were pathogenic on cluster bean and that variation in aggressiveness among all isolates was observed. The fungus caused charcoal rot and developed sclerotia similarly as in our present study in common bean.

Pathogenicity tests with *M. phaseolina* used as inoculum were performed twice on adzuki bean (*Vigna angularis*) and mung bean (*Vigna radiate*) in China and showed strong virulence on inoculated plants (Sun et al. 2016) such as dark-black lesions on the stems, same symptoms as presented in our results. In this study of Sun et al (2016) this is the first report of *M. phaseolina* causing charcoal rot on adzuki bean. These results are consistent by studies previously reported by Rusuku et al. (1997) which showed that *M. phaseolina* used as inoculum in pathogenicity tests caused dark lesion on the stem tissues and cotyledons, and chlorosis in young leaves. Rusuku et al. (1997) suggest that an incessant cropping system of beans or one season rotation with another crop is a common practice in Rwanda and can enhance root rot. Growing other crops at the same time such as sorghum, soybean, and peas in association with bean crops may contribute to fungal diseases caused by pathogens such as Pythium spp., *M. phaseolina* and low levels of occurrence of *Fusarium* species.

L. theobromae **(phenogroup III)** was rather abundant in beans harvested in Boaco and Carazo. Genetic diversification of this species was apparent with two clusters being identified in the phylogenetic analysis (Figure 5, I). Genetic differences correlated geographically, because the isolates from Boaco and Carazo were assigned to different clusters. Common bean seedlings and seedlings of lima bean displayed similar symptoms of dieback, decay and cankers following infection with *L. theobromae*. No difference in pathogenicity was observed between the two genetically distinguishable groups of isolates. Little is known about diseases of common bean caused by *L. theobromae*, although it causes disease in more than 500 plant species (Punithalingan 1976; Punithalingan 1980). *L. theobromae* is a pathogenic fungus occurring in tropical and subtropical regions with capability to colonized plant tissues without any visible symptom of infection. This asymptomatic characteristic is common of endophytic fungi. In addition, this fungus can live as parasite and saprophyte (Rosado et al. 2016; Machado et al. 2014; Dugan et al. 2014; Lima et al. 2013; Punithalingan 1980). The change from a non-pathogenic lifestyle to a disease-causing pathogen may be associated with host stress (Slippers et al. 2007; Lima et al 2013; Rosado et al. 2016). The spores of *L. theobromae* are disseminated

by rain and wind. Use of fungicides and resistant or tolerant cultivars may be helpful to decrease the occurrence of *L. theobromae* infections (Dhingra 1972).

C. cassiicola (**phenogroup IV**) is aggressive facultative parasite sporulating on plant debris (Navas & Subero 1995) with the ability to colonize a wide host range including legumes plants. *C. cassiicola* is considered as a cosmopolitan fungus. It survives in uncultivated soil for more than two years and survives on the soil with or without plant residues (Farr and Rosmman 2015; Lisboa et al. 2016). In our study, two isolates of *C. cassiicola* were detected in a seed lot from Carazo and found to be pathogenic. This fungus has been reported in different crops and countries such as *Navy bean* (China), *Gossypium hirsutum* L (USA) *Cucumis sativus* L (Mexico), *Carica papaya* L (Cuba) and *Momordica charantia* L (China). Pathogenicity tests and characterization of *C. cassiicola* have been topic of the study because it constitutes one of the most important fungal pathogen responsible to cause target spot disease in soybean crops (*Glycine max* L) and cucumber in Brazil under favorable environmental conditions (Teramoto et al. 2013; Teramoto et al. 2011).

Corynespora leaf spot of Balsam pear (*Momordica charantia* L) caused by *C. cassiicola* is the most destructive disease in Korea but also it can infect soybean, sesame oi, cucumber and pepper. The pathogen infects fruits and leaves (Kwon et al. 2005).

Study by Lisboa et al (2016) showed for the first time *C. cassiicola* on *Pueratria phaseoloides* (Fabaceae) causing leaf spot. The symptoms of this fungus comprising reddish brown leaf lesion, chlorotic leaves, necrotic lesions and small brown spot with halos. The damages occur mostly in the reproductive phase. (Brooks 2004). Favorable environmental conditions are needed for infection and disease development including soil temperature range of 15-20 °C. In a dry weather, this fungus is not able to infect leaves and roots (Brooks 2004). The disease cycle is completed in 7-10 days. The efficient dissemination mechanisms (rain and wind) constitute a vast problem for the control of this fungus. The conidia infect leaves and stem.

Phenogroup V belong from genus *Colletrotichum* which comprises seven isolates of *Colletotrichum capsici* (Boaco and Matagalpa) and one isolate of *C. gloeosporioides* (Boaco) detected in the seed lots. ITS sequences of *C. capsici* isolates from Boaco and Matagalpa were identical to each other and to those from pepper in Malaysia, India, and Mexico (Fig. 6; S1 Table, I). In our study, *C. capsici* and *C. gloeosporioides* caused cankers in inoculated lima bean seedlings.

Bean anthracnose is caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara and considered one of the most severe diseases in beans. In navy bean, for instance, infection of 7% of bean seeds is sufficient to cause statistically significant yield losses (Chen et al. 2013). In the absence of susceptible host plants, *Colletotrichum* spp. survive over growing

seasons as mycelia on infested crop residues as saprophytes, or in infected seeds (Schwartz and Hall 2005; Chen et al 2013; Yang et al. 2015). Plants can be infected at any growth stage. Symptoms are more obvious in mature plants and under moist conditions (Anon 1990; Yang et al. 2015).

C. gloesporioides (teleomorph *Glomerella cingulata*) is found to be present in diverse host plants causing several devastating fruit diseases in tropical regions (anthracnose of citrus, fig, mango, olive and avocado, etc.), but also infect vegetables, legumes, cereals, grasses, and ornamental plants. *C. gloesporioides* is considered saprophytic, endophytic, pathogenic and cosmopolitan (Farr et al. 2006; Kumar 2014). In addition, this fungus is considered to be a pathogen of pre-harvest and post-harvest infection (Chowdappa et al. 2012; Nguyen et al. 2011). In Colombia, anthracnose caused by *C. gloesporioides* represents one of the most important disease associated with tomato and mango crops. In mango crops, losses of 60 % reach of the total production (Pardo-De la Hoz et al. 2016).

C. capsici (Syd) Butler and Bisby is a fungal pathogen covering a wide range of different plants growing in tropical areas. There is scarce information of *C. capsici* occurring in legume crops, especially in common bean. However, in a study by Pring et al. (1995) *C. capsici* was reported to infect Cowpea (*Vigna unguiculata*), bean (*Phaseolus vulgaris*) and betel vibe (*Pipper betle*). In cowpea plants *C. capsici* cause brown blotch. Pring et al. (1995) determined virulence of three isolates of *C. capsici* by pathogenicity tests. It was concluded that all isolates were pathogenic showing symptoms similar to those in our results (e.g.rotting, soaked lesions). Anthracnose caused by *C. capsici* represents the major problem on mature fruits provoking severe pre- and post-harvest losses in pepper (Pothita et al. 2005; Sangdee et al. 2011). Anthracnose lesions on chili fruits are caused by *C. capsici* and represent one the most economically important diseases decreasing pepper yields (80-100%) in Thailand (Than et al. 2008). *C. capsici* can survive in and/or on seed (Pernezny et al. 2003).

Studies relating to *C. capsici* in *Capsicum annum*. done in Thailand and India (Sangdee et al. 2011; Deyol et al. 2015) showed high level of pathogenicity and variability among isolates of *C. capsici* in chilli. There is not previous report of *C. capsici* infecting bean crops in Nicaragua. In this study, *C. capsici* is reported for the first time as potential virulent pathogen infecting bean crop.

Two genera, *Penicillium* and *Aspergillus* (**phenogroup VI and VII**), were found to be associated with post-harvest losses in 'INTA Rojo'. *Penicillium* grew out from seed beans of all seed lots and caused mild necrotic symptoms on inoculated bean seedlings. ITS sequences identified the species as *P. citrinum.* A survey of seed lots in Taiwan and Ontario also showed several different *Penicillium* spp. in surface-sterilized beans, albeit not *P. citrinum* (Tseng et al. 1995). Four isolates of *Aspergillus flavus* were obtained from two seed lots

(Estelí and Matagalpa). All of them caused cankers and mild necrosis on inoculated bean seedlings, consistent with a previous study that reported necrosis on roots and stem as well as leaf spots in various legumes caused by this species (Surendranatha et al. 2011). Post-harvest rotting of cereal grains and legumes causes large economical losses as it may destroy 10–30% of the yield—or even higher proportions in developing countries (Agrios 2005). However, the most worrying aspect about *A. flavus* is its potential in many different crops to produce aflatoxins known to be among the most potent carcinogens of biological origin (Probst et al. 2010). *A. flavus* is considered as facultative parasite and saprophyte soil borne fungus responsible to cause diseases in plants, humans and animals (Amaike and Keller 2011). In legume crops such as peanut (Arachis hypogaea) *A. flavus* in the agriculture represent an important pathogen because is responsible to cause disease o*A. flavus* infect grains in the field and usually invade embryos of seeds and decrease germination of infected seed for planting (Klich M A 2007; Michailides and Thomidis 2007). *A. flavus* can produce aflatoxins known to be the most potent carcinogenic substance of biological origin and found to cause mold on grains and legumes. (Agrios 2005; Probst 2010). Thereby, damage is related to agricultural and medical aspects (Gravesen et al. 1994). Commonly, post-harvest diseases are associated to infections of *A. flavus* because aflatoxin contamination of seeds and also due to this fungus surviving and increasing under hot and drought conditions (Cotty 1997; Amaike and Keller 2011). The results suggest that inappropriate crop management in the field and post-harvest practices must be responsible for the rather common occurrence of *Penicillium* and *Aspergillus.*

Fungal isolates from **phenogroup VIII** belong to genus *Diaporthe* sp. Two isolates of *Diaporthe* sp. (synonym *Phomopsis* sp.) [Webster and Weber 2007], one each from Boaco and Estelí, were characterized from the common bean seed lots. When they were used to inoculate lima bean seedlings, white mycelia developed as described in soybeans that suffer from stem and pod blight disease following infection with *Diaporthe phaseolorum* var. *sojae*/*Phomopsis sojae* (Agrios 2005). Furthermore, severe symptoms of necrosis and wilting developed in the inoculated lima bean seedlings were observed. *Diaporthe* spp. are pathogens of many different plant species and cause seed rot, stem cankers, lesions, and pod blight (Thompson et al. 2015), but in common beans they are simply endophytes (Santos et al. 2016). Therefore, it is noteworthy that the two isolates of *Diaporthe* from seed lot of 'INTA Rojo' caused severe disease symptoms in common bean seedlings. Recently, *D. masirevicii* and *D. miriciae* were found associated with cankers on soybean and mung bean plants in Australia (Thompson et al. 2015). Analysis of the ITS regions alone is insufficient to identify the species in the genus *Diaporthe (*Santos et al 2016). It therefore seems warranted to further characterize the pathogenic isolates described in this study using, e.g., multilocus phylogenetic analysis (Santos et al. 2016).

The genus *Diaporthe* sp are phytopathogens, endophytes and saprophytes (Gomes et al. 2013; Udayanga et al. 2011; dos Santos et al. 2015; Thompson et al. 2015). According to Brooks (2004), *Phomopsi*s sp. has been found in plants such as the breadfruit tree (*Artocarpus altilis)* from necrotic spines on fruit and *Carica papaya* L. (from stem canker). Species of *Phomopsis* and *Diapothe* are found in soybeans causing pod and stem blight, as also in other pulse crops (*D. phaseolorum,* anamorph *P. phaseoli*) or stem canker and leaf necrosis of sunflower (*D. helianthi*, anamorph *P. helianthi*) (Webster & Weber 2007). This pathogen causes poor seed quality and low germination. It is responsible for soybean seed rot. Suitable conditions for causing diseases include high temperatures (> 20 °C) and moisture.

Pod and stem blight diseases caused by *Diaporthe phaseolorum* var sojae (*Phomopsis sojae and P. longicolla*) in soybean crops occur well on warm and wet conditions. The infected seeds are showing cracked and shriveled, often covered with mycelium of white color. If the infection is initiated on pods it can infect seeds and as a result cause seed decay. The infection process occurs during or after the yellow pod stage. For this reason, infected seeds are an important source of primary inoculums (AVRDC, 1990). *D. phaseolorum* affecting soybean in susceptible varieties causes yield losses up to 50 % in Europe and North America (Agrios 2005). In Korea, *Phomopsis* sp (Diaporthe) was reported for first time in soybean and found to be pathogenic causing seed decay (Sun et al. 2013).

In their study, morphological and molecular identification and as well as pathogenicity tests were conducted. *Phomopsis* sp (Diaporthe) isolates were identified and pathogenicity tests on hypocotyls showed symptoms such as decay, death of inoculated plant and stem canker. Our results suggested similitude with this study, because *Diaporthe sp* was found infecting bean seeds from Nicaragua. The is the first report of Diaporthe sp on common bean (cv. INTA Rojo) in Nicaragua. Evidence study of *Diaporthe sp* infecting *P. vulgaris* in Nicaragua was not found. However, Diaporthe sp is a seedborne fungus in soybean and beans (Schwartz et al. 2005).

In literature, no evidence for *Diaporthe* sp occurring on bean crop was found. Recently, study done in Australia by Thompson et al. (2015) identified two new fungal isolates of *Diaporthe* sp (*D. masirevicii* and *D. miriciae*) taking place on mung bean plants. In Paraguay, seed decay associated with *Diaporthe* sp in soybean have been not reported but recently study conducted by Mengistu et al. (2014) determined that six from 16 isolates obtained from seeds and pods were pathogenic. The range of infection on pods ranged between 0 to 80 % while infections on seeds ranged between 0 to 27 %. These results are good indicators for our study for the similitude because we showed the importance of this fungus occurring apparently on bean seeds in Nicaragua. Probably, this pathogen has been present on bean crop in Nicaragua as unrecognized species due to lack of good diagnosis.

The strategy using agricultural management for mitigating bean diseases is very important in order to maintain seed quality. The level of infection caused by seed-borne pathogenic fungi can be reduced by good crop management that include the use of certified seed, resistant cultivars, use of potential biological control, crop rotation biological fertilization and avoiding high plant populations (White 1999; Sharma et al. 2015). The use of proper fungicide may be helpful for instance to decrease level of *L. theobromae* infections. In the field, *L. theobromae* can be reduced spraying fungicides such as thiophanate-methyl and carbendazim which inhibit mycelia growth (Diniz et al. 2014). Both fungicides are effective in reducing the fungal infection in mango trees by suppressing the gum exudation, dieback and wilting (Khanzanda et al. 2005). On the other hand, the use of antagonistic microorganisms increases the possibility of handling phytopathological problems at very low costs and without risks to the environment. Antagonists term refers to those organisms that interfere in the survival or development of soilborne pathogens. In nature, there is a continuous interaction between potential pathogens and their antagonists because under natural conditions microorganisms are in a dynamic equilibrium on the surface of the plants. The possibility to apply this technology should be studied as an alternative of safe management of phytosanitary problems caused by soil-dwelling fungi, which parasitize the roots of plants. *Trichoderma* spp. is a fungus present in almost all agricultural soils and other habitats such as decaying wood, plant material, and soil.

Members of the genus *Trichoderma*, include *T. harzianum*, and other species that are part of a healthy soil and can be isolated commonly. Seed treatment using biological control agent (BCA) has been effective to control seed decline and seedling with damping off. Biological control appears to be an appropriate and promising strategy for controlling bean diseases. The efficiency and failure of biological control agents against to infection will depend on whether those courts are stationary or motile, nature of infection, pathogens and environmental conditions (Hall 1996).

4.2. Pathogenicity tests of *Fusarium oxysporum* and development of species-specific primers for detection of pathogenic fungi in common bean (Sub-study II)

Results of pathogenicity tests carried out in two independently repeated experiments on common bean plants (Figure 4, sub-study II) and vegetable cultivars (Figure 5, sub-study II) showed that all the 11 *F. oxysporum* isolates tested caused severe damage in the seedlings, including symptoms of wilting, decay and root rot, and whitish-purple mycelia growing on the stalk. Eventually, infected plants displayed extensive necrotic lesions in the neck and base of the stem. No symptoms were observed in the mock-inoculated controls.

The results of developed specific primers revealed specificity for amplification of the corresponding fungal species.

In this second study, four set of specific primers were designed to amplify *Fusarium* spp (*F. equiseti, F. chlamydosporum* and *F. incarnatum)*; *Corynespora cassiicola*; *Colletotrichum capsici* and *Colletotrichum gloesporiodes* from DNA mycelia and infected bean tissues. The primer set PF1F/PF2R for detection *F. equiseti*, *F. chlamydosporum* and *F. incarnatum* from pure fungal DNA (cultures) and DNA extracted from infected bean tissues generated an amplified fragment of 303 bp (Figure 6, sub-study II).

The developed primer set CcasF/CcasR presented in this work was efficient to detect and amplify a DNA fragment of 177 bp from DNA fungal mycelia and genomic DNA extracted from infected tissue of *C. cassiicola*. This fungus *C. casssiicol*a is an unusual in common bean but regularly found in soybean and pepper crops. However, even our primer set was tested successful in bean tissues and from fungal mycelia, it remains to be tested in bean seeds without previous for detection *C. cassiicola* in very low concentrations (Viana de Sousa et al. 2016).

A primer set for detection *C. capsici* and *C. gloeporioides* was precisely developed for this purpose using pure fungal DNA and DNA extracted from infected bean tissues such as stem and leaves. Consequently, two set of primers CcapF/CcapR (277 bp) and CgF/CgR (100bp), were developed for detection of *C. capsici* and *C. gloesporiodes* in common bean, respectively. The specificity of these two primer sets were checked by PCR assay. The results suggest to us that these two primer sets CcapF/CcapR (277 bp) and CgF/CgR (100bp) are consistent molecular tools for detection of Nicaraguan isolates of *C. capsici* and *C. gloesporiodes.* Based on a result in our study (sub-study I) these two species of *Colletotrichum* were not reported previously in commom bean which means that these primer sets represent a good valuable approach for identification of these pathogenic fungi.

Amplification of other fungal species was not observed using the primers designed for a specific group of fungi indicating that the primers were suitable to differentiate pathogenic fungi of common beans in Nicaragua.

In addition to our designed primers, two pair of primers were tested for detection of *M. phaseolina* and F*. oxysporum*. The primer pair MpKF1/MpKR2 (Babu et al. 2007, Table 2, sub-study II) was used to detect fungal isolates of *M. phaseolina* surveyed from different geographically locations in Nicaragua. Nevertheless, only two of a total of seven isolates were amplified giving an expected product of 350 bp (data not shown). No other tested fungi such as *A. flavus, C. capsici, C. gloesporiodes, C. cassiicola, Diaporthe* sp*, F. equiseti*, *F. oxysporum*, *L. theobromae* and *P. citrinum* were detected with this primer pair. Additionally, it was tested the specificity of the pair of primers designed by Edel et al. (2000) for detection of *F. oxysporum*. Four isolates of *F. oxysporum* obtained from bean plants and one isolate each of F. *equiseti, F. chlamydosporum* and *C. capsici*, all from in Nicaragua, were tested with the

primer pair PFO2/PFO3 (Table 2, sub-study II). The results showed that only two isolates of *F. oxysporum* from Nicaragua were amplified giving a product of the expected size, whereas no PCR product was obtained from other fungi.

4.3. Viruses detected by small-RNA sequencing and RT-PCR (Sub-study III)

Deep sequencing of the small RNAs was used as a tool to detect seed-borne viruses. This method was tested in four Nicaraguan cultivars and landraces of common bean collected from La Compañía, Carazo and as well as, improved varieties from three production areas of common bean of Tanzania. The results of this study using bean samples from Nicaragua and Tanzania indicate to us the occurrence of endornaviruses similar to PvEV-1 and PvEV-2 characterized before in common bean cultivar Black Turtle Soup (Okada et al. 2013). Our results in comparison with those by Okada et al. 2013 suggests major difference in genome organization of PvEV-1 and PvEV-2 if the putative CPS domain is found exclusively in PvEV-1 and the methyltransferase domain found only in PvEV-2.

The occurrence of endornaviruses is vertical via pollination mechanism. This mechanism to help spread of PvEV-1 and PvEV-2 between common varieties, breeding lines and new cultivars growing adjacent. It has not been conducted any study like this in common beans in Nicaragua and Tanzania which could suggests the occurrence of more endornaviruses. PvEV-1 and PvEV-2 have been reported in bean cultivars from the origin center using RT-PCR.

Based on virus symptoms in bean plants from Nicaragua and Tanzania, we decided to test bean plants for begomoviruses infection by universal/degenerate primers (White and Brown 1996). However, begomoviruses were not detected. Our study, suggest us only the presence of PvEVs. Due to the efficient replication of PvEV genome in host cells, endornaviruses are not removed by the antiviral defense. However, they can persist in plants over generations (Okada et al. 2013; Roossinck M. 2011). Endornaviruses are not found to be seed-borne viruses but can disrupt physiology of the plant. Nonetheless, our results in this study suggest that breeding program for resistance genes defends the new released common bean varieties from the most common viruses in Nicaragua and Tanzania.

In the case of bean diseases caused by viruses the best approach to manage these diseases is to use plant resistance and certified seed (healthy seed). These management strategies help to increase yield and quality of common bean. Development of bean cultivars with high levels of viral diseases resistance and genetic improvement has been the goal of many bean-breeding program in Nicaragua, but little progress has been achieved. In Nicaragua some bean improved cultivars have been released by conferring resistance or virus tolerant genes to BCMV, BCMNV and BGYMV. Some of these improved cultivars with resistance genes are SEM46, SEM52, INTA fuerte sequía, INTA cárdenas,

INTA Rojo and the breeding line XRAV-404 (Ferrufino et al. 2013; Jiménez 2012; Jiménez 2013; Jiménez 2014; Beaver et al. 2015). Furthermore, bean viruses transmitted by insect vectors (aphids, beetles and whiteflies) could be controlled by cultural practices e.g. crop rotation, weed removal, and early sowing in the season. These cultural practices help bean plant to reduce the high level of insect populations (INTA 2002, INTA 2009; Buruchara et al. 2010; Kirchner et. 2014; Mwaipopo et al. 2017).

5. CONCLUSIONS AND FUTURE PERSPECTIVES

In the present study detection of seed-borne fungi and viruses infecting bean crops represents an important goal in the national seed system to produce healthy seed and certified seed beans. Little knowledge has been available concerning pathogenic fungi and viruses in common bean in Nicaragua and Tanzania. Thus, the results described in this thesis help to understand better pathogenic infection process in beans and gain knowledge of the locally prevailing seed-borne pathogenic fungi and viruses in bean crops.

First, we can conclude that 133 fungal isolates of seed-associated fungi were obtained from surface-sterilized beans seeds (cv. INTA Rojo) surveyed in the main bean productions areas in Nicaragua.

Second, based on pathogenicity tests, we detected 87 pathogenic fungi which were classified phenotypically to eight distinguishable groups (phenogroups) based on growth and morphological characteristics and further identified by analysis of the ITS1 and ITS2 sequences. The seedborne pathogenic fungi identified in this study were *Fusarium* spp (F. *chlamydosporum, F. equiseti* and *F. incarnatum*), *Macrophomina phaseolina*, *Lasiodiplodia theobromae, Corynespora. Cassiicola*, *Colletotrichum* spp (*C. capsici* and *C. gloeosporioides)*, *Penicillium citrinum*, *Aspergillus flavus* and *Diaporthe* sp (*Phomopsi*s). However, the most common fungi among the pathogenic isolates were Fusarium (*F. chlamydosporum, F. equiseti, F. incarnatum)*, *L. theobromae*, *P. citrinum*, and *M. phaseolina.*

Third, analysis of emergence and growth of bean seedlings showed that germination in seed lots of common bean ('INTA Rojo') from four bean production areas in Nicaragua was less than 40% and as low as 16%. Those seed lots that exhibited better emergence gave rise to a larger proportion of healthy seedlings, whereas poor emergence was associated with a larger proportion of seedlings that emerged but were abnormal and/or were affected by disease-like symptoms.

Four, set of primers were designed for detection of pathogenic fungi such as *Fusarium equiseti*, *F. chlamydosporum, F. incarnatum*, *Colletotrichum capsici*, *C. gloesporiodes* and *Corynespora cassiicola*. The developed sets of

primers amplified DNA only from the corresponding target fungi and might be used to detect those fungi in DNA extracted from fungal mycelia and infected bean plant tissue.

Five, many of the seed-borne pathogenic fungi detected in 'INTA Rojo' were previously unreported in Nicaragua, and reports on occurrence of *F. incarnatum*, *L. theobromae*, *C. cassiicola*, and *Diaporthe*, as seedborne pathogens of common bean are rare elsewhere.

Six, *Phaseolus vulgaris* endornavirus 1 (PvEV-1) and PvEV-2 were identified in bean varieties from Nicaragua and Tanzania. No pathogenic, seedborne viruses were detected in the samples from Nicaragua, but *Cowpea mild mosaic virus* was detected in one region in Tanzania. These results suggest that breeding for resistance and pyramiding resistance genes defends the new CB varieties from the most common viruses in Nicaragua and Tanzania.

Finally, it is to know that common bean is an important crop around world and therefore, has a great potential for genetic and pathological component improvement. Hence, the importance to promote health seeds for keeping good seed quality and to increase production in Nicaragua and Tanzania. In our study, many of the seed-borne pathogenic fungi detected in 'INTA Rojo' were previously unreported in Nicaragua. Reports on occurrence of some fungi such as *F. incarnatum*, *L. theobromae*, *C. cassiicola*, and *Diaporthe* as seedborne pathogens of common bean are rare elsewhere. The incidences of the pathogenic fungi differed between seed lots, which calls for further study to understand better the basis of differences in seed quality which they are linked to handling and storage conditions of seed beans.

In addition, our results provide knowledge base by primer design that will help for seed health inspection and seed certification. However, it is also important to continue studies on epidemiology, ecology, and control of the pathogenic fungi and viruses of common bean in the field and to improve control of the diseases by integrated crop management and use of certified seeds and resistant varieties.

All bean seed farmers are recommended to improve seed cleaning and disease control. The national seed system of bean production could consider testing for seed health from time to time to evaluate efficacy of their efforts to reduce seed infection.

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